Cytogenetic characterization of *Donax trunculus* (Bivalvia: Donacidae) by means of karyotyping, fluorochrome banding and fluorescent *in situ* hybridization[1]

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Abstract

The chromosomes of *Donax trunculus* were analysed by means of Giemsa staining, chromomycin A3 (CA3), DAPI and fluorescent *in situ* hybridization (FISH) with an 18S-5.8S-28S rDNA probe. The diploid number is 38 chromosomes and the karyotype consists of nine pairs of metacentric chromosomes, two pairs of submetacentric-metacentric, seven pairs of submetacentric and one pair of telocentric chromosomes. CA3-positive bands are located on eight chromosome pairs and DAPI treatment resulted in uniform staining. Major ribosomal clusters 18S-5.8S-28S are located on the short arm of one submetacentric chromosome pair.

Introduction

Banding techniques are useful to identify chromosomes and to analyse genomic regions. Some of these techniques have been applied to bivalve molluscs, firstly to investigate the distribution and composition of heterochromatin in mussels (Martínez-Lage, González-Tizón & Méndez, 1994, 1995; Torreiro, Martínez-Expósito, Trucco & Pasantes, 1999; Vitturi, Gianguzza, Colomba& Riggio, 2000), oysters (Li & Havenhand, 1997) and pectinids (Insua, Lopez-Piñon & Méndez, 1998), and secondly to localize the nucleolar organizer regions in mussels (Insua, Labat &Thiriot-Quievreux, 1994; Martínez-Lage et al., 1995; Martínez-Lage, González-Tizón, Ausio & Méndez, 1997a), scallops (Pauls& Affonso, 2000), oysters (Insua & Thiriot-Quiévreux, 1991,1993; Li & Havenhand, 1997; Leitao, Boudry, Labat & Thiriot-Quiévreux, 1999) and cockles (Thiriot-Quiévreux & Wolowicz,1996). Recently, *in situ* hybridization has been used to locate the 18S-5.8S-28S ribosomal genes in some species of mussels (Insua & Méndez, 1998; Torreiro et al., 1999; González-Tizón, Martínez-Lage, Rego, Ausió & Méndez, 2000; Vitturi et al.,2000), two of clams (González-Tizón et al., 2000), one scallop (Insua, Freire & Méndez, 1999) and three of oysters (Zhang, Yu, Cooper & Tiersch, 1999; Xu, Guo, Gaffney & Pierce, 2001). The location of the 5S ribosomal genes, using FISH, was carried out in a scallop (Insua et al., 1998), a cockle (Insua et al., 1999) and in two mussel species (Insua, Freire, Ríos, & Méndez, 2001). The location of telomeric sequences has been reported in seven bivalve species (Guo & Allen, 1997; Martínez-Expósito, Méndez& Pasantes, 1997b; Wang & Guo, 2001; Plohl, Prats, Martínez-Lage, González-Tizón, Méndez & Cornudella, 2002), while the distribution of satellite DNA has been analysed in one oyster (Clabby, Goswami, Flavin, Wilkins, Houghton & Powell, 1996; Wang, Xu & Guo, 2001).

Data on cytogenetics of *Donax trunculus* (Linné, 1758) have been limited to chromosome measurements and to the description of the karyotype in a French population from the Atlantic coast (Cornet & Soulard, 1990a). This showed a chromosome number of 38, composed of nine pairs of metacentric, seven pairs of submetacentric and three pairs of subtelocentric chromosomes. An analysis of a telomeric hexamer repeat in D. *trunculus* was performed by Plohl et al. (2002). This clam is widely distributed along Mediterranean and European Atlantic coasts.

The aim of this work was to analyse the chromosomes of *D. trunculus* from a Spanish Atlantic population by karyotyping, fluorochrome staining and fluorescent *in situ* hybridization(FISH) using an 18S-5.8S-28S rDNA probe.

Materials and methods

Individuals of *Donax trunculus* were collected from Cedeira, Galicia (NW Spain, 43º40'N, 8º3'W). In the laboratory, the animals were fed continuously with a microalgal suspension (*Isochrisis galbana* and *Tetraselmis suecica*) for 10–15 days. Meta-phases were obtained after treatment with colchicine solution (0.005%) for 6–8 h. Gills were dissected, treated twice with 0.075M KCl solution (15 min) and fixed in ethanol: glacial acetic acid (3:1). Fixed cells were dissociated in 45% acetic acid in water and dropped onto heated slides at 43º C. After spreading, meta-phases were stained with 4% Giemsa in phosphate buffer pH 6.8 and photographed with a Nikon Optiphot microscope.

Karyotype was arranged by decreasing size and classified according to the centromeric index, following the nomenclature of Levan, Fredga & Sandberg (1964). Chromosome measurements were taken using the Leica Q-Win 2.2 program (Leica Imaging Systems Ltd). Total chromosome length was measured in 25 metaphases obtained from gill tissue of 25 different individuals. The mean value of the length of the chromosome arms and mean value for total chromosome length were calculated for each chromosome pair. The relative length (100 chromosome length/total haploid length), centromeric index (100 x length of short arm/total chromosome length), mean and SE (SD/no. individuals^{½}) of the relative lengths and centromeric index were also calculated.

Chromomycin A3 (CA3) and DAPI staining were applied following Schweizer (1976, 1980).

FISH was performed using pDm 238 from *Drosophila melanogaster* containing the repeat unit 18S-5.8S-28S rDNA (Roiha, Miller, Woods & Glower, 1981) as a probe. It was labelled with digoxigenin-11-dUTP using a nick translation kit (Roche) according to the manufacturer's instructions. Slides were counterstained with propidium iodide (50 ng/ml antifade), visualized and photographed using a Leica DM-RXA microscope equipped with the appropriate filters.

Results

From 50 individuals of *Donax trunculus*, 285 Giemsa-stained metaphases were analysed, showing that the diploid chromosome number was 38 (Fig. 1). Relative length ranged from 7.79 to 3.65 (Table 1), and the karyotype (Fig.1, Table 1) consisted of nine metacentric pairs (1, 2, 3, 4, 5, 8, 10, 14 and 16), two submetacentric-metacentric (pairs 17 and 18), seven submetacentric (pairs 6, 7, 9, 11, 12, 13 and 15) and one telocentric (pair19).

Staining with fluorochrome CA3 revealed bright positive bands on eight pairs of chromosomes of *D. trunculus* (Figs 2a, 3).Interstitial CA3-positive bands appeared on the long arms of chromosome pairs 1, 3, 5, 11, 14 and 17, while one CA3-positiveband was observed on the short arm of chromosome pair 9. One

subcentromeric CA3-positive band was found on the short arm of chromosome 4. Chromosomes showed uniform DAPI staining (Fig. 2b).

Hybridization with the 18S-5.8S-28S rDNA probe (Figs 2c, 3) revealed the location of ribosomal loci the interstitial region of the short arm of submetacentric chromosome 9. This fluorescent signal is coincident with the CA3-positive band on this chromosome pair.

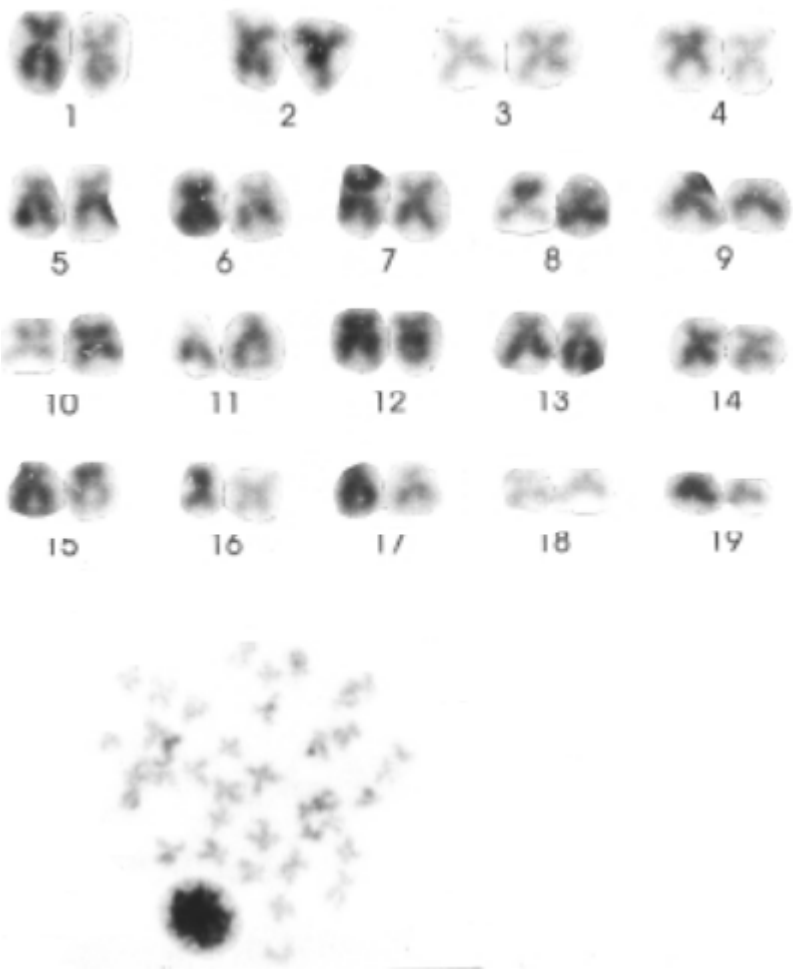

Figure 1. Giemsa-stained metaphase chromosomes and karyotype of *D. trunculus*.Bar = 10 µ m

Discussion

The chromosome number of *Donax trunculus* (2n =38) is the most frequent among the bivalve species previously studied, particularly in the subclass Heterodonta. This number is coincident with that reported by Cornet & Soulard (1990a) in a French population of the species, and with those reported for other species of the superfamily Tellinoidea including *Abra ovata* (Cornet & Soulard, 1987), *Angulus (=Scrobicularia) plana* (Cornet & Soulard, 1989), *Donax variabilis* (Menzel, 1968), *Tellina tenuis* (Cornet & Soulard, 1990b) and *Macoma balthica* (Cornet& Soulard, 1990b). However, there are some differences between the karyotypes of the Galician and French populations, because that of *D. trunculus* from Galicia consists of nine metacentric pairs, two submeta-metacentric pairs, seven submetacentric pairs and one telocentric pair (Fig. 2), while a French population showed nine metacentric, seven submetacentric and three subtelocentric pairs (Cornet & Soulard, 1990a). Any type of chromosome rearrangement could exist between these two populations and other European populations should be examined.

Staining with fluorochrome CA3 has revealed the existence of GC-bands on eight chromosome pairs, at interstitial locations. Results show that *D. trunculus* is a bivalve species, which possesses the highest number of CA3-positive bands found to date, as mussel species *Mytilus edulis*, *M. galloprovincialis* and *M. trossulus* (Martínez-Lage et al., 1994, 1995), *Brachidontes rodriguezi* (Torreiro et al., 1999) and *B. pharaonic* (Vitturi et al., 2000) show CA3-positive bands only on two or three chromosome pairs.

Chromosomes of *D. trunculus* are DAPI-negative, proving that GC-positive regions are AT-negative/DAPI-negative as happens in a great number of animal and plant species, including the mussel species mentioned above.

Nowadays, the most appropriate method to detect rDNA cistrons is the fluorescent *in situ* hybridization, because it allows the physical location of ribosomal genes to be determined independently of transcription activity. Although the FISH technique has been extensively employed in many animal and plant species, it has rarely been applied to bivalves. In *D. trunculus*, Ag-NOR staining (data not shown) and *in situ* hybridization with an 18S-5.8S-28S rDNA probe located the ribosomal clusters on the interstitial region of the short arms of the submetacentric chromosome pair 9. Comparing the location of ribosomal genes 18S-5.8S-28S in *D. trunculus* with other bivalves, only the mussel *Mytilus californianus* (Martínez-Lage et al., 1997; González-Tizón et al., 2000) showed ribosomal loci in a subterminal location, although some venerid species also show ribosomal loci in these regions (unpublished data). Most of bivalves analysed by these means tend to show the ribosomal clusters at telomeric regions. The present results show the association of GC-rich heterochromatin and ribosomal genes, as described in other bivalve species (Martínez-Lage et al., 1994, 1995; Torreiro et al.,1999; Vituri et al., 2000).

In conclusion, these results provide new information about cytogenetic characteristics of D. *trunculus* and contribute to increase the small number of bivalve species analysed to date.

Table 1. Relative lengths and centromeric indices of *Donax trunculus* chromosomes

Pair	**RL**	**CI**	**Type**
1	7.79 ± 0.32	47.82 ± 0.95	m
2	6.80 ± 0.12	44.14 ± 1.17	m
3	6.55 ± 0.10	46.33 ± 1.42	m
4	6.23 ± 0.06	44.74 ± 1.63	m
5	5.82 ± 0.06	43.90 ± 0.83	m
6	5.69 ± 0.16	31.30 ± 1.72	sm
7	5.61 ± 0.10	30.45 ± 1.14	sm
8	5.57 ± 0.11	45.94 ± 1.00	m
9	5.25 ± 0.07	32.32 ± 1.52	sm
10	5.15 ± 0.16	44.18 ± 0.98	m
11	5.09 ± 0.07	31.54 ± 1.25	sm
12	4.91 ± 0.07	32.25 ± 1.64	sm
13	4.81 ± 0.14	31.78 ± 1.50	sm
14	4.68 ± 0.17	43.51 ± 0.96	m
15	4.44 ± 0.08	30.01 ± 2.02	sm
16	4.19 ± 0.22	43.07 ± 1.25	m
17	4.07 ± 0.09	34.68 ± 1.36	sm/m
18	3.71 ± 0.12	34.69 ± 1.87	sm/m
19	3.65 ± 0.26	0.50 ± 0.10	t

Abbreviations: RL, relative length; CI, centromeric index;m, metacentric; sm, submetacentric; t, telocentric

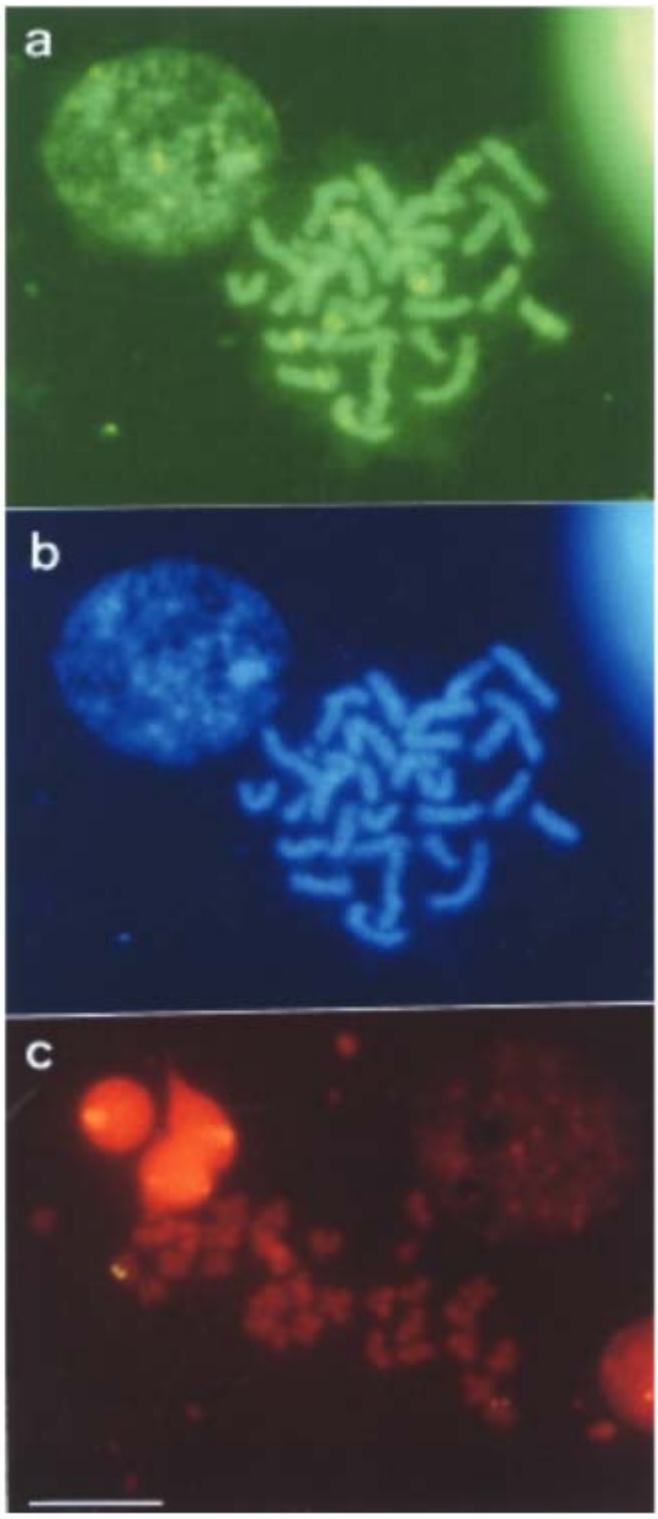

Figure 2. Metaphase chromosomes of *Donax trunculus.* **A.** Chromomycin A3staining. **B.** DAPI staining. **C.** Fluorescent *in situ* hybridization with a rDNA probe. Bar = 10 µm

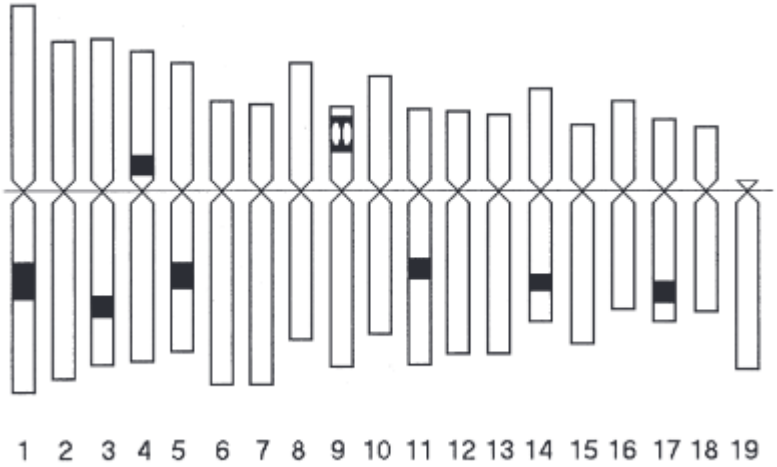

Figure 3. Idiogram of kariotype of *Donax trunculus* based on chromosome morphology and size. Chromomycin A3 bands (black) and 18S-5.8S-28SrDNA clusters (white circles).

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